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(54) Title: PECTIN COMPOSITION AS FAT REPLACER AND EMULSIFIER

(57) Abstract

There is provided a fat replacer comprising a pectin composition, wherein the pectin composition comprises at least a population of pectin which is covalently cross linked.

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PECTIN COMPOSITION AS FAT REPLACER AND EMULSIFIER

The present invention relates to a use of a composition. In particular, the present invention relates to a use of a pectin composition.

Pectin is an important commodity in industry. For example, it can be used in the food industry as a thickening or gelling agent, such as in the preparation of jams or fruit systems for yoghurt, or for the stabilisation of acidified milk suspensions.

Pectin is a structural polysaccharide commonly found in the form of protopectin in plant cell walls. The backbone of pectin comprises α-1-4 linked galacturonic acid residues which are interrupted with a small number of 1,2 linked α-L-rhamnose units. In addition, pectin comprises highly branched regions with an almost alternating rhamno-galacturonan chain. These highly branched regions also contain other sugar units (such as D-galactose, L-arabinose and xylose) attached by glycosidic linkages to the C3 or C4 atoms of the rhamnose units or the C2 or C3 atoms of the galacturonic acid units.

Some of the carboxyl groups of the galacturonic residues are esterified (e.g. the carboxyl groups are methylated). Typically esterification of the carboxyl groups occurs after polymerisation of the galacturonic acid residues. However, it is extremely rare for all of the carboxyl groups to be esterified (e.g. methylated). Usually, the degree of esterification will vary from 0-90%. If more than 50% of the carboxyl groups are esterified then the resultant pectin is referred to as a "high ester pectin" ("HE pectin" for short) or a "high methoxyl pectin". If less than 50% of the carboxyl groups are esterified then the resultant pectin is referred to as a "low ester pectin" ("LE pectin" for short) or a "low methoxyl pectin". If 50% of the carboxyl groups are esterified then the resultant pectin is referred to as a "medium ester pectin" ("ME pectin" for short) or a "medium methoxyl pectin". If the pectin does not contain any - or only a few - esterified groups it is usually referred to as pectic acid.

Pectin has also found other uses in the food industry, for example use as a fat replacer or as an emulsifier.

WO-A-96/03440 discloses pectin derived from sugar beet which is modified using a laccase enzyme. The phenolic groups of the sugar beet are modified such that a cross linked gel is provided. It is taught in WO-A-96/03440 that the gel may be used in foodstuffs as thickening, stabilising, setting or viscosity regulating agents or that the gel may be used in medicinal or agricultural applications.

EP-A-0 656 176 discloses a calcium sensitive pectin. Calcium is added to the pectin to form ionic bonds between the pectin. The ionic bonding results in the formation of a gel. The gel is dehydrated, dried and milled. EP-A-0 656 176 teaches that the milled pectin gel may be used for *inter alia* replacement of fat in a foodstuff.

With increased demand for low fat containing food products, there is a desire to provide further materials which may be used to fully or partially replace fat in food products.

The present invention aims to overcome the problems of the prior art.

According to a first aspect of the present invention there is provided a fat replacer comprising a pectin composition, wherein the pectin composition comprises at least a population of pectin which is covalently cross linked.

According to a second aspect of the present invention there is provided use of a pectin composition as a fat replacer, wherein the pectin composition comprises at least a population of pectin which is covalently cross linked.

Yet further we have found that a pectin composition comprising at least a population of pectin which is preferably cross linked may be incorporated in a foodstuff and act both as a fat replacer and an emulsifier. This dual function has not previously been recognised.

Thus, according to a third aspect of the present invention there is provided use of a pectin composition as an emulsifier and a fat replacer in a foodstuff.

It has also been surprisingly found that a pectin composition comprising at least a population of pectin which is covalently cross linked may also act as an emulsifier.

According to a fourth aspect of the present invention there is provided an emulsifier comprising a pectin composition wherein the pectin composition comprises a least a population of pectin which is covalently cross linked.

According to a fifth aspect of the present invention there is provided use of a pectin composition as an emulsifier wherein the pectin composition comprises a least a population of pectin which is covalently cross linked.

According to a sixth aspect there is provided a process for preparing a foodstuff, the process comprising the step of incorporating a pectin composition in the foodstuff, wherein the pectin composition comprises at least a population of pectin which is covalently cross linked, as a fat replacer to partially or completely replace fat.

We have found that contrary to the teaching of the prior art which discloses that only ionically bound i.e. calcium gelled, pectins may be used as fat replacers or as emulsifiers, one may use a covalently linked pectin as a fat replacer or as an emulsifier or as both.

Yet further we have found that a covalently linked pectin, which may be used as a fat replacer or as an emulsifier or as both, when combined with an emulsifier provides a creamy mouthfeel which is improved when compared that which would be expected from a combination of the covalently linked pectin and emulsifier. In other words, the present invention provides an emulsifying composition having a synergistic improvement in its organoleptic properties.

The presence in a pectin composition of "population of pectin which is cross linked" may be determined in accordance with Protocol I, Protocol I is recited at the end of the Examples section (*infra*).

The presence in a pectin composition of a "population of pectin which is covalently

cross linked" may be determined in accordance with Protocol II, Protocol II is recited at the end of the Examples section (*infra*).

It will be appreciated by a person skilled in the art that the term "covalently cross linked" is analogous with the term "oxidatively cross linked" and with the term "oxidatively gelled".

The term "fat replacer" means a material which may be incorporated in a foodstuff partially or completely in place of fat, preferably in place of triglycerides. The material may be incorporated in the foodstuff in place of the incorporation of fat, may be incorporated in the foodstuff in place of fat which is already present or may be incorporated in the foodstuff to reduce the concentration of fat based on the amount of foodstuff. The term "fat replacer" is analogous with the term "fat mimetic".

Whilst the term "pectin" is used to describe the various aspects of the present invention, it is possible for any polysaccharide to be used in place of the pectin. Therefore, in its broadest aspect, the present invention provides each of the aspects of the present invention described herein but wherein the pectin composition is substituted by a polysaccharide composition comprising at least a population of polysaccharide which is cross linked, preferably which is covalently cross linked.

Preferably, the polysaccharide of the broad aspect of the present invention is a polysaccharide containing phenolic groups, such as sugar beet pectin or arabinoxylan.

Alternatively, in a broad aspect the present invention provides the above aspects wherein pectin is substituted by a pectin isostere. Thus the present invention provides

- a fat replacer comprising a composition, wherein the composition comprises at least a population of a pectin isostere which is covalently cross linked.
- use of a composition as a fat replacer, wherein the composition comprises at least a population of a pectin isostere which is covalently cross linked.
- use of a composition as an emulsifier wherein the composition comprises a least a population of a pectin isostere that is covalently cross linked.
- use of composition comprising a pectin isostere as an emulsifier and a fat replacer

in a foodstuff.

- an emulsifier comprising a composition wherein the composition comprises a least a population of a pectin isostere which is covalently cross linked.
- a process for preparing a foodstuff comprising incorporating a composition, wherein the composition comprises a least a population of pectin isostere which is covalently cross linked, as a fat replacer to partially or completely replace fat.

The term "pectin" includes fractions of pectin, one or more compounds from the class of compounds known as pectins, and derivatives thereof.

The term "derivatives thereof" includes derivatised pectin and degraded pectin (such as partially degraded pectin) and modified pectin.

Preferably, the emulsifier is an emulsifier having emulsifying properties in accordance with Protocol III.

Preferably, the pectin composition is obtainable by or is obtained by contacting a pectin material with a cross-linking agent to provide at least a population of pectin which is covalently cross linked.

Preferably, the cross-linking agent is selected from enzymes, chemical cross-linking agents, including oxidants e.g. peroxide, persulfate; mixtures and derivatives thereof. The cross-linking agent may be selected from those disclosed in J.-F. Thibault et al. (3) (a combination of a peroxidase and hydrogen peroxide); WO 93/10158 (a peroxide, such as hydrogen peroxide, and an oxygenase, preferably a peroxidase); and FR-A-2 545 101 (hydrogen peroxide and peroxidases).

Preferably the cross-linking agent is an enzyme .

Preferably the enzyme is selected from laccases (EC 1.10.3.2) [which are oxidases (i.e. enzymes employing molecular oxygen as acceptor) capable of catalysing oxidation of phenolic groups], phenol-oxidising oxidases (EC 1.10.3.1), glucoseoxidases, hexoseoxidases, mixtures and derivatives thereof.

Preferably, the enzyme is a laccase.

Laccases are obtainable from a variety of microbial sources, notably bacteria and fungi (including filamentous fungi and yeasts). Preferably, the laccases is obtainable from or is obtained from a strain selected from *Aspergillus*, *Neurospora* (e.g. *N. crassa*), *Podospora*, *Botrytis*, *Collybia*, *Fomes*, *Lentinus*, *Pleurotus*, *Trametes* [some species/strains of which are known by various names and/or have previously been classified within other genera; e.g. *Trametes villosa* = *T. pinsitus* = *Polyporus pinsitis* (also known as *P. pinsitus* or *P. villosus*) = *Coprinus* (e.g. *C. plicatilis*], *Psatyrella*, *Myceliophthora* (e.g. *M. thermophila*), *Schytalidium*, *Phlebia* (e.g. *P. radita*; see WO 92/01046, or *Coriolus* (e.g. *C. hirsutus*; see JP 2-238885).

A preferred laccase in the context of the invention is obtainable from or is obtained from *Myceliophthora thermophila*.

Typically, the pectin material is contacted with the cross-linking agent, for example a laccase, in the presence of atmospheric air. This will normally suffice to ensure an adequate supply of oxygen for oxidation, although forcible aeration of a reaction mixture with air, or possibly even substantially pure oxygen, may be advantageous under certain conditions.

When the cross linking agent is an enzyme, the enzyme will typically be contacted with the pectin material wherein the pectin material is in a solution. Before contacting the enzyme with the pectin material it will generally be preferable to adjust the pH of the solution to a value equal to, or in the vicinity of, the optimum pH for the enzyme in question. Preferably the pH of the solution 2.5 to 6.0.

When the enzyme is laccase, preferably the amount of laccase contacted with the pectin material is in the range of 0.01-1000 kLAMU per kg of pectin material, preferably 0.05-100 kLAMU/kg of pectin material, and will typically be in the range of 0.1-100 kLAMU per kg of pectin material. LAMU (1 kLAMU = 1000 LAMU) is the unit of laccase activity as defined in Protocol IV. For ease of reference, Protocol IV is recited

at the end of the Examples section (*infra*).

In a preferred embodiment, the pectin material is contacted with laccase in an amount of 1-10 kLAMU/kg pectin. Preferably the pectin material is in a 3% solution. Preferably, the solution is saturated with oxygen.

Preferably, the pectin material and/or pectin composition comprises a population of pectin which is acetylated. Preferably, the pectin material and/or pectin composition comprises a population of pectin which contains ferulic acid groups. Preferably, the pectin material and/or pectin composition comprises a population of pectin which is acetylated and which contains ferulic acid groups.

Preferably, the pectin composition has a ferulate content of from approximately 0.4-0.9%.

Preferably the pectin material and/or the pectin composition is obtainable from or is obtained from members of the plant family *Chenopodiaceae* (which includes beets, spinach and mangewurzels). More preferably, the pectin material and/or the pectin composition is obtainable from or is obtained from beets, yet more preferably, sugar beets.

Preferably the pectin material and/or the pectin composition contains at least a population of pectin which have phenolic substituents. More preferably, the pectin material and/or the pectin composition contains at least a population of pectin which have phenolic substituents derived from cinnamic acid.

The pectin composition may be extracted from the pectin material under a wide range of conditions. It has been found that the following extraction condition, either above or in combination are preferred:-

- Extraction temperature: 60-90°C
- Extraction pH: 1.5-3.0

Preferably, the pectin composition has a content of neutral sugars of from 10 to 30 wt.%.

The pectin material and/or the pectin composition and/or the cross linked pectin population may be obtainable or may be obtained by fractionation of a pectin or a pectin composition.

Preferably the pectin composition is obtainable or is obtained by selecting sugar beet pulp, and extracting the pectin composition by a process comprising the steps of:

Extraction

- provide sugar beet pulp
- mix with an acid solution of nitric acid
- adjust the ratio of water to pulp to approximately 1:20
- adjust the pH, if necessary, to 1.5-3.0
- heat to a temperature of 60-90°c for approximately 1 to 6 hours.

Precipitation

- the solution is passed through an ion exchange to remove calcium
- adjust the pH or, alternatively adjust pH after next step
- added to isopropyl alcohol to precipitate the pectin.
- precipitated pectin is dried and milled to a required particle size.

Cross Linking

- the milled pectin is solubilised to form a 0.5-3% solution at a pH of 3-6
- cross binding agent, for example laccase enzyme, in an amount of 10 LAMU per gram of pectin in solution, is added. The solution is allowed to stand for several hours, normally overnight.
- the cross linked pectin may be then isolated by precipitation as described above.

In the above preferred process the precipitation, drying and milling of the pectin may be omitted. Cross binding agent may be contacted directly with the extracted pectin material after ion exchange. Thus the pectin composition may be obtainable or may be obtained by selecting sugar beet pulp, and extracting the pectin composition by a

process comprising the steps of:

Extraction

- provide sugar beet pulp
- mix with an acid solution of nitric acid
- adjust the ratio of water to pulp to approximately 1:20
- adjust the pH, if necessary, to 1.5-3.0
- heat to a temperature of 60-90°c for approximately 1 to 6 hours.

Ion Exchange

- the solution is passed through an ion exchange to remove calcium
- adjust the pH, for example to 3-6.

Cross Linking

- cross binding agent, for example laccase enzyme, in an amount of 10 LAMU per gram of pectin in solution, is added. The solution is allowed to stand for several hours, normally overnight.
- the cross linked pectin may be then isolated by precipitation as described above.

The covalent cross-linking ability of the sugar beet pectin appears to be dependent on the form of the extraction process.

As mentioned above, in a broad aspect, the present invention provides each of the aspects of the present invention wherein the pectin composition is substituted by a polysaccharide. In this broad aspect the polysaccharide (and/or a polysaccharide material from which it may be derived) may be obtainable from or may be obtained from one of arabinoxylans, preferably arabinoxylans containing phenolic substituents derived from cinnamic acid such as those obtainable from cereals; heteroxylans, preferably bran including wheat bran and maize bran.

The pectin composition for use in the present invention may comprise a population of pectin that is calcium sensitive and/or a population of pectin that is calcium insensitive.

The term calcium insensitive means a population of pectin which has a calcium sensitivity index (CF) equal to 1. A Protocol for determining calcium insensitivity may be found on page 57 of WO-A-97/03574 (the contents of which are incorporated herein by reference). For ease of reference, this Protocol is recited at the end of the Examples section (*infra*) as Protocol V.

The proportion of the pectin composition that is calcium insensitive may be determined in accordance with the method of Protocol VI. Protocol VI is recited at the end of the Examples section (*infra*).

The pectin material and/or the pectin composition and/or the cross linked pectin population may comprise any one or more of a low ester pectin, a medium ester pectin or a high ester pectin.

Modification and/or control of the degree of esterification pectin material and/or the pectin composition and/or the cross linked pectin population may be required. The degree of esterification of the pectin material and/or the pectin composition and/or the cross linked pectin population may be modified and/or controlled by use of a pectin methylesterase (EC 3.1.1.11), otherwise referred to as a PME. PMEs de-esterify HE pectins to HE pectins with a lower DE , to LE pectins or pectic acids.

The use of PMEs is advantageous because PME activity produces free carboxyl groups and free methanol. The increase in free carboxyl groups and thus the degree of esterification can be easily monitored by automatic titration.

For example, the degree of esterification of the pectin material and/or the pectin composition and/or the cross linked pectin population is modified and/or controlled in accordance with a process described in International Patent Application No PCT/IB98/00673, filed 24 April 1998.

The ionically cross linked pectin population of a pectin composition in accordance with the present invention is preferably a calcium sensitive high ester pectin, or a low ester pectin comprising an amidated pectin and/or a low ester pectin having a degree of

esterification of 28-38%).

A Protocol for determining the degree of esterification and acetylation of a pectin may be found in Food Chemical Codex, edition 4 (the contents of which are incorporated herein by reference). For ease of reference, this Protocol is recited at the end of the Examples section (*infra*) as Protocol VII.

For high ester calcium sensitive pectins, calcium cross-linking can take place because sites on the pectin molecules have low degree of esterification such that calcium may interact by electrostatic/ionic means. This property is usually described as calcium sensitivity. High ester calcium sensitive pectin is capable of forming micro gels with calcium. These gels have the property that they carry an amount of water and additionally it is possible to separate micro gels from excess water just by centrifugation.

Low ester pectins cross-linked with calcium form a different type of micro gel. These pectins give reversible gels, meaning that if a given gel is disrupted one may obtain gel particles or pieces. However, when mechanical treatment of the gel stops, the gel starts to re-build because of the re-establishment of hydrogen bonds. Gel compositions comprising an amidated pectin are then again different from gels with low ester conventional pectin (LC pectin). The former gel composition will melt down on heating of the gel to 50-60°C, whereas much more energy (e.g. heating to greater than 100°C) is needed to melt down an LC pectin gel.

The covalent cross-linked pectin population of a pectin composition in accordance with the present invention, may be cross-linked through feroyl groups. In this aspect, preferably the degree of esterification of the pectin material which is cross-linked is in the range of 45-60%. As described above, the degree of esterification can then be modified by adding methoxyl groups or by de-esterifying with acid or with enzymes, for example pectin esterases. It is known however that a non-cross-linked sugar beet pectin can not be used as a gelling agent unless acetyl groups are removed, and it might be necessary to do so in combination with the esterification or the de-esterification to obtain an effect. By modifying the sugar beet pectin with one or more of the above mentioned tools it is possible to adjust a covalently cross-linked composition to a given food system with the

objective to obtain an additional effect such as ionic and/or hydrogen bond formation and/or hydrophobic interaction.

In the aspect of the present invention wherein the pectin composition comprises a population of ionically cross-linked high ester pectin the proportion of the composition which is calcium sensitive pectin is preferably greater than 50%, more preferably, greater than 60%, more preferably greater than 70%, more preferably greater than 80%, more preferably greater than 90%, yet more preferably greater than 95%. In a highly preferred embodiment a citrus based pectin fraction is isolated which has a proportion of calcium sensitive pectin of approximately 100%. Such fraction could be obtained in accordance with the teachings of EP-A-0664300 or Danish Patent Application No. 120/98. Subsequent enzyme treatment with a pectin esterase de-methylating a high ester pectin in a block-wise manner may be performed according to WO-A-97/03574, filed 12 July 1996. Alternatively, a pectin material and/or pectin composition may be obtained simply by selection of suitable citrus raw materials.

In the aspect wherein an ionically cross-linked pectin population is obtained from sugar beet pectin, the following steps may be necessary:

- optionally, esterification of the sugar beet pectin
- de-acetylation
- deesterification by pectin methyl esterase, de-esterifying in a block-wise manner

In one aspect, the pectin composition may comprise a population of pectin which is covalently cross-linked and a population of pectin which is ionically cross-linked. In this aspect, the pectin composition and/or pectin material is preferably obtained by or obtainable by de-acetylating and, optionally also de-esterifying, a sugar beet pectin.

Preferably, the pectin composition has a water binding capacity of about 20 to about 70. Water binding capacity is measured in accordance with WO 96/03440(5) and WO 97/27221(6).

Preferably, the pectin composition is in particulate form. Preferably, the particulate pectin composition has an average particle size of no greater than 75-100 μm.

The cross-linked pectin of the present invention may be combined with an emulsifier. As discussed above the covalently linked pectin of the present invention may be combined with a second emulsifier to form a composition for use an emulsifier. The emulsifier composition provides a creamy mouthfeel which is improved when compared that which would be expected from a combination of the covalently linked pectin and the second emulsifier – the present invention provides an emulsifying composition having a synergistic improvement in its organoleptic properties.

The second emulsifier may be selected from monoglycerides, diglycerides, derivatives and mixtures thereof.

Preferably, the emulsifier and/or fat replacer of the present invention or as provided by the present invention is incorporated in a foodstuff.

Thus, in a seventh aspect, the present invention provides a foodstuff comprising a fat replacer as described herein.

In a eighth aspect, the present invention provides a foodstuff comprising an emulsifier as defined herein.

Preferably, the foodstuff is selected from dairy products (such as milk products, fresh cheese, sour cream and ice cream), low fat spread, mayonnaise, yoghurt, sauces, meat and meat products, poultry products, fish products, fruit spreads, bakery products including bakery fillings, margarine, reconstituted fruits, jams, fruit preparations, fruit fillings, ripples, fruit sauces, stewed fruit, coffee whitener, instant fruit dessert, confectionery (such as marsh mallow), and fine foods (such as dressings including salad dressings; ketchup, vinaigrette dressings and soups) The foodstuff may be a beverage. The beverage may be a drinking yoghurt, a fruit juice, a beverage concentrate or a fruit based beverage.

The term "foodstuff" can include food for human and/or animal consumption.

The present invention may be used in the preparation of a starting reagent or an intermediate in the preparation of a foodstuff.

Alternatively, present invention may be used in the preparation of a foodstuff itself.

The pectin composition may be incorporated in materials other than foodstuffs. For example, the pectin composition may be incorporated in cosmetics (such as creme) and pharmaceuticals.

The preparation of a pectin composition in accordance with the present invention and its use in a composition in accordance with the present invention will now be described.

EXAMPLES

In the examples the following analytical methods were used.

Analytical methods

The contents of methanol and acetic acid were determined after alkaline deesterification in an alcoholic suspension by HPLC (9).

The amount of galacturonic acid was determined by the m-hydroxybiphenyl method (10).

These analyses made it possible to calculate the degree of esterification and acetylation.

The content of ferulic acid was determined spectrophotometrically at 375nm on freshly prepared solutions of pectin in 0.15M glycin buffer pH10 using a molar extinction coefficient of 31600 (11).

Sodium content was determined by AES after dissolving the sample in diluted nitric acid.

The individual neutral sugars were determined by HPLC after hydrolysis in 1M sulphuric acid at 100°C for two hours(12).

The viscosity-average molecular weight of pectins were determined using the following method.

- A 0.90% pectin solution is made in a 1% sodium hexametaphosphate solution pH 4.5.
- The duration of the fall period in second of the ball in the pectin solution and in the sodium hexametaphosphate solution is measured by a Hoppler viscometer.
- The method is then repeated.
- The molecular weight is calculated as the difference between the fall time in the two solutions multiplied by 200,000 and divided with the fall time in the sodium hexametaphosphate solution.

The ability to cross-link was tested in a 2% aqueous solution of the pectin by adding 20 LAMU laccase (5,6) per g pectin while stirring. That the time elapsed before the gel was formed is a mark of the oxidative gelling properties. Furthermore, the formed gel was inspected visually or by Bohlin rheometer to determine the consistency.

The free swelling capacity and the retention capacity of the cross-linked product were measured (5,6) .

Extraction of Pectin Material (1-8)

The raw materials used were Fibrex 595 and Fibrex 608, both commercial dried sugar beet pulp and fresh, frozen sugar beet pulp as well as dried sugar beet pulp (available from Danisco Sugars, Denmark).

The pectin material was extracted with nitric acid at different pH (1.0/1.5/2.4), at different temperatures (95°C/85°C/70°C) and for different time periods (1 hour/4 hours). The ratio between pulp and solvent was 1:20. In the pectin extracts with pH 1

and 1.5 the pH was adjusted to pH 4 before filtration, and ion exchange. Because of the low molecular weight of the pectin the extracts were concentrated to a pectin content of approx. 4% before the pectin material was precipitated in 80% isopropanol. The precipitate was dried and milled to an average particle size of less than 200 μm.

The analysis of the pectin material is shown in Tables 1 to 4 below

Both pectin material extracted from commercially available Fibrex and pectin material extracted from dried or wet sugar beet pulp were analysed.

The types of pectin extracted from the commercial Fibrex had a rather low molecular weight while the yields were high. In contrast when dried or wet pulp was used as raw material, pectin with a higher molecular weight was obtained but the yields were rather low.

Extraction at conditions of greater than pH 1 and/or less than 90°C were acceptable. Operation outside these conditions degraded the pectin, both the molecular weight, the degree of esterification and acetylation decreased.

Extraction at pH 1.5 and 85°C gave a pectin with a ferulate content of 0.6-0.7%, while the pectin extracted at 70°C had a ferulate content of 0.8-0.9%. Molecular weight was not affected. The ferulate content was directly correlated to the amount of neutral sugars and arabinose. This was expected as the ferulate groups are mainly attached to the arabinose side chains.

A higher content of galacturonic acid was obtained from the pulp from the sugar beet pulp when using a higher temperature.

Preparation of Cross-Linked Pectin Composition

A solution comprising 3% pectin material as prepared above was provided. The pH was adjusted to 5.5. The solution was saturated with oxygen and a laccase (5,6) was added at a dosage of 10 LAMU per g pectin material. Stirring and aeration was

continued until gelling occurred. After 16 hours the gel was washed several times with water after which the gel was dewatered with isopropanol.

A fibrous precipitate was obtained. The pectin composition product was dried and ground to an average particle size of 250 μm.

The results of the analysis of the cross linked pectin compositions are given in Tables 1 to 4 below.

TABLE 1

Raw Material	Extraction Conditions *)	% Yield	Cross-link Ability **)	Molecular Weight	% ferulate	% galacturonic acid	% neutral sugars	% methanol	% acetic acid	% Na
Fibrex 608	70°C, pH 2.4 4 hours, HCl, -	25	+ >30 min.	12000	0.73	44	28	4.1 (53%)	5.6 (38%)	approx. 1-2
Fibrex 595	70°C, pH 2.4 4 hours, HCl, -	25	++ >30 min.	13000	0.80	45	24	4.4 (56%)	6.9 (46%)	approx. 1-2
Fibrex 595	70°C, pH 2.4 4 hours, -	-	++ >30 min.	14000	0.83	47	27	4.4 (53%)	6.8 (43%)	1.3
Fibrex 595	85°C, pH 1.5 1 hour, +	32	+++ 30 min.	12 000	0.68	44	17	3.9 (50%)	5.8 (39%)	4.0
Fibrex 595	85°C, pH 1.0 1 hour, +	30	+++ >30 min.	9400	0.56	56	14	2.7 (27%)	2.8 (15%)	6.2
Fibrex 595	95°C, pH 1.5 1 hour, +	33	++ >30 min.	8000	0.63	45	13	2.9 (37%)	4.4 (29%)	4.8
Fibrex 595	95°C, pH 1.0 1 hour, +	28	-	6100	0.53	61	9	2.4 (22%)	1.6 (8%)	5.5
Reference sugar beet pectin		-	+++++ <5min	51000	0.53	60	13	5.2 (49%)	5.0 (25%)	1.5

*) Extraction temperature, extraction pH, extraction time, + neutralisation / - neutralisation. Nitric acid was used for extraction when nothing else is indicated.

**) Judgement of the gel :+++++ is the highest judgement. The time given is the gelation time.

TABLE 2

Raw material	Extraction conditions	Crosslink ability	% neutral sugars	% rhamnose	% arabinose	% galactose	% glucose
Fibrex 608	70°C, pH 2.4 4 hours, HCl	+ >30min	28	0.3	24	3.3	0.4
Fibrex 595	70°C, pH 2.4 4 hours, HCl	++ >30min	24	0.3	18	4.6	0.6
Fibrex 595	70°C, pH 2.4 4 hours	++ >30	27	0.3	20	5.7	0.7
Fibrex 595	85°C, pH 1.5 1 hour	+++ 30min	17	0.5	7.3	7.6	1.4
Fibrex 595	85°C, pH 1.0 1 hour	+++ >30min	14	0.3	5.7	6.9	1.4
Fibrex 595	95°C, pH 1.5 1 hour	++ >30min	13	0.3	5.7	5.5	1.4
Fibrex 595	95°C, pH 1.0 1 hour	-	9	0.2	3.4	5.1	0.8
Reference sugar beet pectin		+++++ <5min	13	0.5	4.9	7.2	0.3

TABLE 3

Raw material	Extraction conditions *)	% Yield	Cross-link ability **)	Molecular weight	% ferulate	% galacturonic acid	% neutral sugars	% methanol	% acetic acid	% Na
Fibrex 595	85°C, pH 1.5 1 hour, +	-	+++ 30min	12000	0.68	44	17	3.9 (50%)	5.8 (39%)	4.0
Wet beet pulp	70°C, pH 2.4 4 hours, -	10	++++ 5min	34000	0.86	42	27	4.6 (62%)	5.8 (41%)	1.5
Wet beet pulp	85°C, pH 1.5 1 hour, +	20	++++ 10min	34000	0.69	61	13	4.7 (44%)	5.9 (29%)	3.1
Dry beet pulp	85°C, pH 1.5 1 hour, +	16	++++ 20min	30000	0.68	61	10	4.6 (43%)	5.7 (28%)	3.5
Dry beet pulp	70°C, pH 2.4 4 hours, -	7	++++ <5min	37000	App. 0.8	42	app.20 -30			app. 1-2
Reference sugar beet pectin	-	-	+++++ <5min.	51000	0.53	60	13	5.2 (48%)	5.0 (24%)	1.5

*) Extraction temperature, extraction pH, extraction time, + neutralisation / - neutralisation. Nitric acid was used for extraction when nothing else is indicated.

**) Judgement of the gel +++++ is the highest judgement. The time given is the gelation time.

TABLE 4

Raw material	Extraction Conditions	Cross-link ability	% neutral sugars	% rhamnose	% arabinose	% galactose	% glucose
Fibrex 595	85°C, pH 1.5 1 hour	+++ 30min	17	0.5	7.3	7.6	1.2
Wet beet pulp	70°C, pH 2.4 4 hours	++++ 5min	27	0.3	22	4.5	0.2
Wet beet pulp	85°C, pH 1.5 1 hour	++++ 10min	13	0.4	5.6	7.1	0.3
Dry beet pulp	85°C, pH 1.5 1 hour	++++ 20min	10	0.4	2.6	6.7	0.4
Dry beet pulp	70°C, pH 2.4 4 hours	++++					
Reference sugar beet pectin		+++++ <5min	13	0.4	5.6	7.1	0.3

Application Tests

Pectin material was obtained from sugar beet. The pectin material was cross linked as described above to produce a pectin composition. The yield was 366 g which corresponded to approx. 60%. The product had a free swelling capacity on 48 and a retention capacity on 45.

The pectin composition was used in the following applications.

Example 1 - Ice Cream

An ice cream containing the pectin composition in accordance with the present invention and no fat, was compared to an ice cream with fat and one without. The dosage was 0.5%. The recipes are shown in Table 5.

The maximal dosage of the pectin composition was 0.5% otherwise the thickness of ice cream mix increased to an extent such that pumping became difficult.

The ice cream produced had increased the creaminess compared to the two references, both the product with fat and without, i.e. it worked as a fat replacement. It did not have any effect on the body or the cold/warm feeling in the mouth.

TABLE 5

COMPOSITION	**1**	**2**	**3**
Component	Weight %		
Water	67.39	67.26	67.23
Cocowar HCNO 31	3.00		
Skimmed milk powder	12.60	12.60	12.60
Sucrose	12.00	12.00	12.00
Glucose syrup solids, 32 DE	4.21	6.84	7.37
CREMODAN® SE 709 VEG	0.80	0.80	0.80
Pectin composition		0.50	

(Cross linked sugar beet pectin)			
Total	**100.00**	**100.00**	**100.00**
Total fat	3.13	0.13	0.13
Total MSNF	11.97	11.97	11.97
Total solids	31.90	31.90	31.90

Process:

1. Melt the fat at 50°C
2. Mix liquid ingredients at 20-22°C
3. Mix dry ingredients
4. Mix liquid and dry ingredients at 20-22°C
5. Add fat and increase the temperature to 30°C
6. Pasteurise at 78°C
7. Cool to 25-30°C
8. Ageing overnight in ice water
9. Stir
10. Measurement of viscosity
11. Freeze to 100% overrun

Example 2 - Ice cream (Fat Replacing Effect)

The fat replacing effect of the cross-linked sugar beet pectin was tested in ice cream. The dosage was in the range of 0.4% to 0.8%. The recipes are shown in the table. Fat-containing ice cream (3% and 8%) together with Slendid 200 and Fibruline (a commercial fat replacer based on inulin) were used as references. The samples were evaluated and given scores from 0-6 for the creaminess, where the 8% fat-containing ice cream had a creaminess of 3. Furthermore meltdown was measured. The cross-linked sugar beet pectin gave an increased creaminess, i.e. it had fat replacing properties:

TABLE 6

Composition (per cent)	1	2	3	4	5	6	7	8	9
Water	66.28	66.38	63.94	66.21	66.24	66.25	66.24	66.24	66.24
Coconut HCNO 31		3.00	8.00						
Skimmed milk powder	12.60	12.60	11.30	12.60	12.60	12.60	12.60	12.60	12.60
Sucrose	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00
Glucose syrup solids, 32 DE	8.32	5.37	4.21	7.99	7.76	7.55	7.76	7.76	
CREMODAN® SE 709 VEG	0.80	0.65	0.55	0.80	0.80	0.80	0.80	0.80	0.8
Cross-linked sugar beet pectin				0.40	0.60	0.80			
Sugar beet pectin							0.60		
Slendid 200								0.60	
Fibruline									7.00
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Total fat	0.13	3.13	8.11	0.13	0.13	0.13	0.13	0.13	0.13
Total MSNF	11.97	11.97	10.74	11.97	11.97	11.97	11.97	11.97	11.97
Total dry matter	32.80	32.85	35.40	32.89	32.87	32.87	32.87	32.87	33.10

Process

1. Melt fat at approx. 50°C
2. Mix liquid ingredients at 20-22°C
3. Mix dry ingredients
4. Add fat - increase temperature to 30°C
5. Pasteurise at 78°C/2-3 minutes
6. Homogenise at 78°C
7. Cool to 25-30°C
8. Ageing overnight in ice water (1-2°C)
9. Stir
10. Freezing with 100% overrun

TABLE 7

No	Composition	Creaminess	Melt down (%)
1	Non fat	1	72
2	3% fat	3	8
3	8% fat	3	1
4	Non fat + 0.4% CLSBP	4	57
5	Non fat + 0.6% CLSBP	4	37
6	Non fat + 0.8% CLSBP	5	9
7	Non fat + 0.6% SBP	4	46
8	Non fat + 0.6% Slendid	2	28
9	Non fat + 7% Fibruline	2	45

Creaminess is evaluated,

0 = no creaminess,

3= creaminess of a full fat ice cream and

6 = max creaminess.

Melt down = %-melted ice cream after 60 minutes.

The cross linked sugar beet pectin (CLSBP) and the sugar beet pectin (SBP) give outstanding creaminess and the melt down is delayed. The ice cream with CLSBP or SBP is thus similar to the fat-containing ice creams.

Example 3 - Ice Cream

Emulsifying effect and combined effect of fat replacement and emulsifying effect

The pectin composition was tested in a recipe without emulsifier to evaluate both the fat replacement and the emulsifying effect. The ice cream is evaluated as described above.

TABLE 8

Composition (per cent)	1	2	3	4	5	6	7	8
Water	67.07	66.86	66.87	66.87	66.87	67.02	67.04	67.02
Coconut HCNO 31	-	-	-	-	-	3.00	3.00	3.00
Skimmed milk powder	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60
Sucrose	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00
Glucose syrup solids, 32 DE	7.28	7.90	7.68	7.47	7.68	4.53	4.32	4.53

Emulsifier part of CREMODAN® SE 709 VEG	0.55	-	-	-	-	-	-	-
Cross-linked sugar beet pectin	-	0.40	0.60	0.80	-	0.60	0.80	-
Beet pectin	-	-	-	-	0.60	-	-	0.60
Stabiliser part of CREMODAN® SE 709 VEG	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Total fat	0.13	0.13	0.13	0.13	0.13	3.13	3.13	3.13
Total MSNF	11.97	11.97	11.97	11.97	11.97	11.97	11.97	11.97
Total dry matter	32.30	32.25	32.25	32.25	32.25	32.25	32.25	32.25

Process

1. Melt fat at approx. 50°C
2. Mix liquid ingredients at 20-22°C
3. Mix dry ingredients
4. Add fat - increase temperature to 30°C
5. Pasteurise at 78°C/2-3 minutes
6. Homogenise at 78°C
7. Cool to 25-30°C
8. Ageing overnight in ice water (1-2°C)
9. Stir
10. Freezing with 100% overrun

Results

E = Emulsifier part of the CREMODAN 709 VEG

S = Stabiliser part of the CREMODAN 709 VEG

Tested as emulsifier

TABLE 9

Sample	Creaminess	Melt down (%)
3% fat with E+S	3	8
3% fat +S + 0.6% CLSBP	1 - 2	38
3% fat +S + 0.8% CLSBP	1 - 2	6
3% fat +S + 0.6% SBP	2	54

Test of combined effect (fat replacement and emulsifying effect)

TABLE 10

Sample	Creaminess	Melt down (%)
Non fat E+S	1	72
Non fat+S	0	85
Non fat +S + 0.4% CLSBP	1	51
Non fat +S + 0.6% CLSBP	1	21
Non fat +S + 0.8% CLSBP	1	18
Non fat +S + 0.6% SBP	0	56

Ice cream with 3% fat and the CREMODAN 709 VEG has medium creaminess, while the CLSBP and the SBP give moderate creaminess.

In non-fat ice cream with the CLSBP the creaminess is as good as non-fat ice cream with emulsifier. The stabiliser system alone or the SBP alone do not give any creaminess. Again the melt down of the non-fat ice creams with CLSBP are superior to the ones of emulsifier and stabiliser system.

Comparison of all ice cream tests suggests a special outstanding effect concerning creaminess when CLSBP or SBP is combined with emulsifier.

Example 4 - Mayonnaise

Two products of which the compositions were identical with the exception of 0.2% pectin composition in accordance with the present invention added in one of the products. The recipes are shown in Table 11.

The pectin composition in accordance with the present invention was tested in a 10% mayonnaise with a dosage of 0.2%. It gave a slightly increased viscosity, but no significant difference between the two products.

TABLE 11

Ingredients	1	2
Phase 1, Starch		
Water	25.00	25.00
Oil	5.00	5.00
Sugar	5.00	5.00
Ultratex 4	3.00	3.00
Starch 570		
Swelygel 105	2.00	2.00
Vinegar	1.50	1.50
Wine vinegar	1.50	1.50
Lactic acid 50%	0.45	0.45
SUM	**43.45**	**43.45**
Phase 2, Acid		
Lemon juice	0.50	0.50
SUM	**0.50**	**0.50**
Phase 3, Hydrocolloids		
Water	41.22	41.42
Onion powder	0.05	0.05
Maltodextrine 15	3.50	3.50
Titanium dioxide	0.20	0.20
Salt	1.50	1.50
Xanthan gum	0.50	0.50
Alginate FD175	0.18	0.18
Soya oil	5.00	5.00
Calcium sorbate	0.20	0.20
Skim milk powder	2.50	2.50
Mustard	1.00	1.00
Pectin composition (Cross linked sugar beet pectin -C 2099/53)	0.20	
SUM	**56.05**	**56.05**
TOTAL	**100.00**	**100.00**

Process

- Run phase 1 as a cold process on the Koroman. Very little mechanical treatment. Stirring for 2 minutes at rotation 8. Then drained into a bucket.
- More mechanical treatment is required for phase 3, mix till all ingredients are fully dissolved.
- Phase 2 is added after 4 minutes.
- Lastly add phase 1 and run at low rotation for 2 minutes at rotation 8.
- Pack the product.

Example 5 - Low fat spread

The test was performed with a pectin composition in accordance with the present invention added at three different dosages.

With a dosage of 0.5% no protein was added. In the low fat spread with 1.0% pectin composition 1% whey powder was added as protein source while at 1.5% level SMP (Skim Milk Powder) at a dosage of 1 % was added. As references Slendid 200 a calcium gelled, ionically cross linked pectin available from Hercules Inc. Delaware, USA and a mixture of pectin known as LFS 100* was used. Recipes are shown in Table 12.

At all dosage levels the low fat spread was stable except for sample 7 (reference LFS 100 with SMP) which separated. The spread containing pectin composition of the present invention provided good mouth feel

* LFS 100 is a high ester citrus pectin available from Danisco Ingredients, Denmark.

TABLE 12

Product type:	Low fat spread								
J.No. :1116	1	2	3	4	5	6	7	8	9
WATER PHASE:									
Water	58.2	58.2	58.2	56.2	56.2	56.2	55.7	55.7	55.7
Salt	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Whey powder				1	1	1			
SMP							1	1	1
Pectin composition (Cross linked sugar beet pectin -C 2099/53)		0.5			1.5			2.0	
LFS 100	0.5			1.5			2.0		
LFS 560									2.0
Slendid 200			0.5			1.5			
Potassium sorbate	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
EDTA	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015
pH	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
WATER PHASE total	60	60	60	60	60	60	60	60	60

FAT PHASE:									
Soya 41	25	25	25	25	25	25	25	25	25
Soya oil	75	75	75	75	75	75	75	75	75
FAT IN PARTS total	100	100	100	100	100	100	100	100	100
FAT total	39.5	39.5	39.5	39.5	39.5	39.5	39.5	39.5	39.5
DIMODAN® CP	0.5	0.5	0.5						
DIMODAN® OT				0.5	0.5	0.5	0.5	0.5	0.5
PPM β-carotene	4	4	4	4	4	4	4	4	4
FAT PHASE total	40	40	40	40	40	40	40	40	40
RECIPE total	100	100	100	100	100	100	100	100	100
FLAVORING no. (w or. o)									
GRINDSTED™ 3385 (o)	0.01	0.01	0.01						
GRINDSTED™ 2807 (w)	0.01	0.01	0.01				0.01	0.01	0.01
GRINDSTED™ 2873 (o)				0.01	0.01	0.01			
GRINDSTED™ 3507 (w)				0.01	0.01	0.01			
GRINDSTED™ 4452 (o)							0.01	0.01	0.01
Process conditions									
Capacity kg / h	50	50	50	20	20	20	20	20	20

Process

1. Blend the salt and chosen Stabiliser System and dissolve in the water, while agitating vigorously. Heat to 40°C and add the whey powder and K-sorbate. Adjust pH. It may be necessary to pasteurise the water phase or emulsion.
2. Melt the fat blend and adjust the temperature to 40°C. Add the β-carotene.
3. Heat the emulsifier with some of the oil in a ratio of 1:5 to a temperature (60-65°C) which is 5-10°C higher than the melting point of the emulsifier. When this blend is completely melted and well stirred, add it to the remaining heated fat blend, stirring continuously.
4. Add the flavourings.
5. Make the emulsion by adding the water phase slowly to the fat phase, stirring vigorously. Emulsion temperature 50°C.
6. Crystallise and knead vigorously in a tube chiller Outlet temperature approx. 12°C.

TABLE 13

SFC values of fat blend (IUPAC method)	Fat blend (spread used at approx. 5-10°C)
5°C	23
10°C	19
20°C	9
30°C	2
35°C	0
40°C	0
Slip melting point °C (AOCS 3-25 method)	28.6

Example 6 - Low fat spread

The pectin composition was tested in different recipes of low fat spread. In non-milk recipes and in whey based recipes the composition works equally well as the products such as GRINDSTED™ LFS 150, Gelatine and Slendid 200. The composition is not able to stabilise hard fat low fat spread as is also the problem with other products for the low fat spreads industry.

Below is given an example of the use of the composition in a 40% low fat spread with skim milk powder. The performance of the composition is compared to other products in the marked for the low fat spread industry.

TABLE 14 - Different stabilisers for LFS

Trial no 1	1	2	3	4	5	6	7	8	9
WATER PHASE:									
Water	54.7	56.7	56.2	56.7	56.2	56.7	56.2	56.7	56.2
Salt	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Milk powder	1	1	1	1	1	1	1	1	1
Gelatine HB 240	3								
Slendid 200		1	1.5						
GRINDSTED™ LFS 150				1	1.5				
Sugar beet pectin (SBP)						1	1.5		
Crosslinked SBP								1	1.5
Potassium sorbate	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

EDTA	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015
pH	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
WATER PHASE total	60	60	60	60	60	60	60	60	60
FAT PHASE:									
Soya 41	25	25	25	25	25	25	25	25	25
Soya oil	75	75	75	75	75	75	75	75	75
FAT IN PARTS total	100	100	100	100	100	100	100	100	100
FAT total	39.5	39.5	39.5	39.5	39.5	39.5	39.5	39.5	39.5
DIMODAN® OT	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
PPM ß-carotene	4	4	4	4	4	4	4	4	4
FAT PHASE total	40	40	40	40	40	40	40	40	40
RECIPE total	100	100	100	100	100	100	100	100	100
FLAVOURING no. (w or. o)									

Stability was evaluated after one day's tempering at 5°C.

Evaluation: 10- very stable and smooth, 8- stable, separates when worked intensively, 6-separates when worked, 4- separates when work slightly, 2-separates and 0 = separated

TABLE 15

Trial 1	1	2	3	4	5	6	7	8	9
Gelatine HB 240	3								
Slendid 200		1	1.5						
Grindsted ™ LFS 150				1	1.5				
SBP						1	1.5		
CLSBP								1	1.5
Evaluation	8	2	5	5	8	4	5	6	8

In a dosage of 1.5 % the CLSBP is stabilising the low fat spreads equally well as the gelatine and the GRINDSTED™ LFS 150.

Example 7 – Yoghurt

Fat replacing and stabilising effect

The fat replacing and the stabilising effect of the CLSBP was tested in yoghurt. The dosage was 0.10%, 0.15%, 0.20%, 0.30% 0.50%. The recipes are shown in the Table 16.

TABLE 16

Composition	1	2	3	4	5	6	7	8	9	10
Whole milk 3.5%	99.40	28.50								
Skimmed milk		70.80	97.20	96.90	96.90	96.90	96.90	96.90	96.90	96.90
Skimmed milk powder			2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
TS-D 1492	0.60	0.70	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Slendid 200				0.15						
CLSBP					0.10	0.15	0.20			
SBP								0.10	0.15	0.20
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Total fat	**3.48**	**1.07**	**0.12**	**0.12**	**0.12**	**0.12**	**0.12**	**0.12**	**0.12**	**0.12**

Process

1. Mix dry ingredients while stirring
2. Heat to 65°C
3. Homogenise at 65°C/200 bar
4. Pasteurise at 90°C/10 minutes
5. Cool to 42°C
6. Add 0.02% B3 culture (Christian Hansen A/S)
7. Fermentation at 42°C to pH 4.4
8. Stir and cool to 25°C
9. Fill
10. Store cool

Results

The CLSBP and the SBP gave stabilising effect at 0.10% dosage and instability at a dosages higher than 0.15%. A creaminess similar to the yoghurt was obtained as well.

Example 8 - 10% Thousand Island Dressing

The fat replacing effect of cross-linked sugarbeet pectin was investigated in a dressing by replacing 20% fat with cross-linked sugarbeet pectin. The dosage range was 0.2-0.5% and a full fat dressing was used as a reference. A fat replacement effect was observed.

TABLE 17

Ingredients	1	2	3	4	5	6	7	8	9
Water	40.65	60.30	60.10	60.30	60.10	60.10	61.10	61.10	61.30
Oil	30.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Salt	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sugar	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
GRINDSTED™ FF 2112							3.80	3.80	3.80
GRINDSTED™ FF 2110	0.95	1.00	1.00	1.00	1.00	1.00			
Sugar beet pectin cross-linked		0.30	0.50				0.20		
Sugar beet pectin native				0.30	0.50			0.20	
Slendid 200						0.50			
Vinegar 7%	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Tomato paste	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Granulated onion	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Onion flour	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Granulated Garlic	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Chop. pickled cucumber	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
egg yolk	3.50	3.50	3.50	3.50	3.50	3.50			
Potassium sorbate	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
SUM	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Process (1-6)

- Mix water, salt and sugar
- Blend starch and stabiliser with some of the oil and add it to the water phase
- Mix until all ingredients are fully dissolved
- Emulsify the rest of the oil into the water phase
- Add vinegar and mustard
- Pack

Process (7-9)

- Mix water, salt and sugar
- Blend starch and stabiliser with some of the oil and add it to the water phase
- Mix until all ingredients are fully dissolved
- Emulsify the rest of the oil into the water phase
- Add vinegar and mustard
- Heat the water phase to 85°C and hold for 4 minutes
- Cool the water phase to 20°C
- Pack

Example 9 - Range Dressing

The emulsifying effect of the cross-linked sugarbeet pectin was evaluated by comparing the composition with PGA and GRINDSTED™ FF2104. The dressing was viscous, and therefore it was difficult to distinguish between the emulsifying effect and the viscosity effect, but a stable product was obtained.

TABLE 18

Ingredients	10	11	12	13	14	15	16
Water	32.76	32.76	32.56	32.36	32.56	32.36	32.86
Oil	38.00	38.00	38.00	38.00	38.00	38.00	38.00
Buttermilk	15.00	15.00	15.00	15.00	15.00	15.00	15.00
White wine vinegar 10%	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Buttermilk powder	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sugar	2.00	2.00	2.00	2.00	2.00	2.00	2.00
salt	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Liquid egg yolk	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Xanthan gum	0.24		0.24	0.24	0.24	0.24	0.24
PGA	0.10						
GRINDSTED™ FF 2104(TS-F 032)		0.34					
Onion granulate	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vinegar 10%	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Garlic granulate	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Flavour blue q265-15-1	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Black pepper granulate	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Sugar beet pectin cross-linked			0.30	0.50			
Sugar beet pectin native					0.30	0.50	
Beta carotene (ppm)							
SUM	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Process

- Water, sugar, salt, buttermilk, flavour and spices is mixed
- Stabilisers, buttermilk powder are blended with some of the oil and added
- Egg yolk is added.
- Oil is emulsified into the water phase.
- Vinegar added
- pH is adjusted to 3.5 in all trails, with phosphoric acid.

Example 10 - Cosmetic o/w emulsion

The pectin composition was tested as emulsifier in a cosmetic o/w emulsion. The concentration of the cross-linked pectin was 2.5%.

TABLE 19

Ingredients	Concentration (w/w)
Water phase:	
Water	81.2
Sorbitol	4.3
Urea	1.0
Pectin composition	2.5
Oil phase:	
Hexyldecanol, Hexyldecyl Laurate	4.0
Dicaprylyl ether	4.0
Caprylic/capric triglyceride	3.0

The cross-linked sugar beet pectin alone was able to emulsify the cosmetic emulsion. Furthermore it provided a fine skin feel and some greasiness.

Summary of tests with cross-linked sugar beet pectin.

Cross-linked sugar beet pectin (CLSBP) is an excellent and competitive fat replacer in ice cream. In Europe the inulin based product, Fibuline is often used as replacer of fat in low and no fat ice cream but the fat replacing effect is obtained at a quite high dosage of the Fibruline, 7 %. In the further testing of possible emulsifying effect by the SBP and the CLSBP the emulsifier was excluded from the recipe and it was found that the creaminess is obtained as a combined effect of SBP or especially CLSBP and the emulsifier. It should be noted here that alone the emulsifier is not able to give creaminess. Also the traditional solution for low and no fat ice cream, namely the presence of emulsifier, stabilisers and Fibruline, gives poor creaminess. CLSBP was found to be superior to SBP in tests where the emulsifier part was excluded from the recipe.

In dressings and low fat spreads the CLSBP and SBP are effective as stabilisers of the

emulsion. CLSBP is more effective than SBP.

In cosmetic emulsions the CLSBP was able to emulsify the emulsion and to provide superior creaminess and skinfeel.

Sourcing of Ingredients

Ingredients referred to herein may be obtained from the following sources

Name	Commercial name	Supplier
Coconut HCNO 31	KOKOWAR 31	Århus Olie
Skimmed mild powder		MD Foods
Sucrose	Sukker	Danisco Sugar
Glucose syrup solids, 32 DE	C*dry 01934	Cerestar
CREMODAN® SE 709 VEG		DANISCO CULTOR
Cross-Linked sugar beet pectin CLSB CSBP SBP		DANISCO CULTOR
Slendid 200		Hercules
Oil	COLZAO	Århus Olie
Grindsted FF 2112		DANISCO CULTOR
Grindsted FF 2110		DANISCO CULTOR
Vinegar 7%	7% Estragoneddike	Lagerberg
Tomato paste		TH. Schultz
Granulated onion		Danske Krydderier
Onion flour		Danske Krydderier
Granilated Garlic		Danske Krydderier
Chop. pickled cucumber		TH. Schultz
Egg Yolk	Blomme m. 6% salt	Sanovo Foods
Potassium sorbate	Potassium sorbate powder	Nutrinova
Butter Milk		MD Foods
White wine vinegar 10%		Lagerberg
Buttermilk powder		MD Foods
Xanthan gum		DANISCO CULTOR
PGA	PGA 800	DANISCO CULTOR
Grindsted FF 2104		DANISCO CULTOR
Onion granulate		Danske Krydderier
Flavour blue q265-15-1		DANISCO CULTOR
Black pepper granulate		Danske Krydderier
Grindsted LFS 150		DANISCO CULTOR
LFS 150	Grindsted LFS 150	DANISCO CULTOR
LFS 120	Grindsted LFS 120	DANISCO CULTOR
Soya 41	SHOGUWAR 41	ÅRHUS OLIE
Soya oil	SHOGUN	ÅRHUS OLIE
DIMODAN ® OT		DANISCO CULTOR
ß-CAROTENE	BC-3000 OSS	Chr. Hansen
Soya 35	SHOGUWAR 35	ÅRHUS OLIE
Coconut oil	KOKONEUTREX	ÅRHUS OLIE

Gelatine HB 240		SKW
Urea	Carbamid	Superfos Biokemi
Hexyldecanol	Cetiol PGL	Bröste
Dicaprylyl ether	Cetiol OE	Bröste
Caprylic/capric triglyceride	RYLO TG 50	DANISCO CULTOR

PROTOCOL I

CROSS LINKED

A cross-linked pectin will, without added calcium, form micro gels, i.e. the pectin will not be dissolved. In contrast, native pectin without added calcium will dissolve.

A 1% solution/suspension is made by adding pectin to be studied to water (50°C) whilst stirring vigorously. The solution/suspension is left over night at room temperature.

If formed micro gels are detected after centrifugation in Eppendorf tubes at 12800xg, the tested composition comprises a population of pectin which is cross-linked.

PROTOCOL II

COVALENTLY CROSS LINKED

To 5ml of 2% suspension of cross-linked material 15 ml 1% sodium hexametaphosphate pH 4.5 is added. The mixture is stirred for two hours. If the product is ionically cross-linked, the micro gels will disappear while the micro gels from the covalent cross-linked will remain. The micro gels can be detected as described above in Protocol I.

PROTOCOL III

EMULSIFIER

A model system as described below is used for testing emulsifying properties.

Model O/W emulsion

Oil phase (20%)

100% refined rape seed oil, which equals 20% in the mixture.

Water phase (80%)

X% emulsifier, which equals X x 0.8 % in the mixture (100-X)% water, which equals (100-X) x 0.8% in the mixture.

The model system is studied as follows

- The emulsifier is dispersed in hot water (50°C)
- Add the oil phase to the water while stirring vigorously
- Mix with Ultra Turrax for 60 seconds at 9500 rpm and then treat in a laboratory size homogeniser at 400 bar
- Cool and degas
- The emulsifying effect is monitored by
  - 1. visual inspection and/or
  - 2. microscopy and/or
  - 3. Particle size measurement by Malvern

Tween -8- is used as reference. A model system is considered to exhibit an emulsifying effect if the phases do not separate within one week, i.e. the particle size does not considerably change.

PROTOCOL IV

DETERMINATION OF LACCASE ACTIVITY (LAMU)

Laccase activity as defined herein is determined on the basis of spectrophotometric measurements of the oxidation of syringaldazin under aerobic conditions. The intensity of the violet colour produced in the oxidation reaction is measured at 530 nm.

The analytical conditions are: 19 µM syringaldazin, 23.2 mM Tris buffer, pH 7.5, 30°C, reaction time 1 minute. 1 laccase unit (LAMU) is the amount of enzyme that catalyses the conversion of 1 µM of syringaldazin per minute under these conditions.

PROTOCOL V
CALCIUM SENSITIVITY INDEX (CF)

Calcium sensitivity is measured as the viscosity of a pectin dissolved in a solution with 57.6 mg calcium/g pectin divided by the viscosity of exactly the same amount of pectin in solution, but without added calcium. A calcium insensitive pectin has a CF value of 1.

4.2 g pectin sample is dissolved in 550 ml hot water with efficient stirring. The solution is cooled to about 20°C and the pH adjusted to 1.5 with 1N HCl. The pectin solution is adjusted to 700 ml with water and stirred. 290 g of this solution is measured individually into 2 viscosity glasses. 10 ml water is added to one of the glasses (double determinations) and 10 ml of a 250 mM $CaCl_2$ solution is added to the other two glasses under stirring.

50 ml of an acetate buffer (0.5 M, pH about 4.6) is added to both viscosity glasses under efficient magnetic stirring, thereby bringing the pH of the pectin solution up over pH 4.0. The magnets are removed and the glasses left overnight at 20°C. The viscosity's are measured the next day with a Brookfield viscometer. The calcium sensitivity index is calculated as follows:

$$CF = \frac{\text{Viscosity of a solution with 57.6 mg } Ca^{2+}\text{/ g pectin}}{\text{Viscosity of a solution with 0.0 mg } Ca^{2+}\text{/ g pectin}}$$

PROTOCOL VI
METHOD OF DETERMINING CISP CONTENT

A pectin composition was fractionated into a calcium sensitive fraction (CSP) and a calcium insensitive fraction (CISP) in accordance with the method below

- Dissolve 1 % sugar free pectin in water
- Adjust pH to 4.5 with a solution of 10 % Na_2CO_3
- Make a fractionation solution of 60 mM $CaCl_2$/16% IPA/water
- Measure 20 ml fractionation solution in a 80 ml centrifugation glass

- Inject about 20 grams pectin solution into the fractionation solution, the precise amount to be noted
- Centrifuge at 5000 rpm for 20 minutes
- Separate
- Add 30 ml fractionation solution, diluted in a ratio of 1:1 with water
- Mix the sedimented gel and the diluted fractionation solution with a spatula and centrifuge at 5000 rpm for another 5 minutes
- Repeat this procedure twice
- Dissolve the gel with a few drops of 3 N HCl and stir with a spatula
- Add 60 ml 60 % IPA/3% HCl/37% H_2O to the dissolved gel. Mix and centrifuge at 5000 rpm for 5 minutes
- Wash out chloride from the material with 60 ml 60 % IPA four times. Each wash is mixed, centrifuged and separated
- The CSP fraction is transferred to a low weight plastic petri dish (which has been weighed on beforehand)
- Dry at 40 °C over night

the CISP content can be calculated

PROTOCOL VII

DEGREE OF ESTERIFICATION (%DE) AND ACETYLATION

Degree of amide Substitution and Total Galacturonic Acid in the Pectin Component

Weigh 5 g of the sample to the nearest 0.1 mg. and transfer to a suitable beaker. Stir for 10 min with a mixture of 5 mL of 2.7 *N* hydrochloric acid, and 100 mL of 60% ethanol. Transfer to a fritted-glass filter tube (30- to 60-mL capacity), and wash with six 15-mL portions of the same hydrochloric acid-60% ethanol mixture, followed by 60% ethanol until the filtrate is free of chlorides. Finally, wash with 20 mL of ethanol, dry for 2.5 h in an oven at 105°, cool and weigh. Transfer exactly one-tenth of the total net weight of the now ash-free, dried sample (representing 0.5 g of the original unwashed sample) to a 250-mL conical flask, and moisten the sample with 2 mL of ethanol. Add 100 mL of recently boiled and cooled distilled water, stopper, and swirl occasionally until a complete solution is formed. Add 5 drops of phenolphthalein TS,

titrate with 0.1 *N* soldium hydroxide and record the results as the initial titre (V_1).

Add exactly 20 mL of 0.5 *N* sodium hydroxide, stopper, shake vigorously, and let stand for 15 min. Add exactly 20 mL of 0.5 *N* hydrochloric acid, and shake until the pink color disappears. Titrate with 0.1 *N* sodium hydroxide to a faint pink color that persists after vigorous shaking; record this value as the saponification titre (V_2).

Quantitatively transfer the contents of the conical flask into a 500-mL distillation flask fitted with a Kjeldahl trap and a water-cooled condenser, the delivery tube of which extends well beneath the surface of a mixture of 150 mL of carbon dioxide free water and 20.0 mL of 0.1 *N* hydrochloric acid in a receiving flask. To the distillation flask add 20 mL of a 1 in 10 sodium hydroxide solution, seal the connections, and then begin heating carefully to avoid excessive foaming. Continue heating until 80 to 120 mL of distillate has been collected. Add a few drops of methyl red TS to the receiving flask, titrate the excess acid with 0.1 *N* sodium hydroxide, and record the volume required, in mL, as *S*. Perform a blank determination on 20.0 mL of 0.1 *N* hydrochloric acid, and record the volume required, in mL, as *B*. Record the amide titre (B - S) as V_3.

Transfer exactly one-tenth of total net weight of the dried sample (representing 0.5g of the original unwashed sample), and wet with about 2mL of ethanol in a 50-mL beaker. Dissolve the Pectin in 25 mL of 0.125 *M* sodium hydroxide. Let the solution stand for 1 h, with agitation, at room temperature. Transfer quantitatively the saponified Pectin solution to a 50-mL volumetric flask, and dilute to volume with distilled water. Transfer 25.0 mL of the diluted Pectin solution to a distillation apparatus, and add 20 mL of Clark's solution, which consists of 100 g of magnesium sulfate heptahydrate and 0.8 mL of sulfuric acid and distilled water to a total of 180 mL. The distillation apparatus consists of a steam generator connected to a round-bottom flask to which a condenser is attached. Both steam generator and the round-bottom flask are equipped with heating mantles. Start the distillation by heating the round bottom flask containing the sample. Collect the first 15 mL of distillate separately in a measuring cylinder. Then start the steam supply and continue distillation until 150 mL of distillate has been collected in a 200-mL beaker. Quantitatively combine the distillates, titrate with 0.05 *M* sodium hydroxide to pH 8.5, and record the volume

required, in mL, as *S*.

Perform a blank determination using 20 mL of distilled water. Record the required volume, in mL, as *B*. Record acetate ester titre (*S*-*B*) as V_4.

Calculate the degree of amidation (as the percent of total carboxyl groups) by the formula

$$100 - [V_3/(V_1 + V_2 + V_3 - V_4)].$$

Calculate mg of galacturonic acid by the formula

$$19.41\ (V_1 + V_2 + V_3 - V_4).$$

The mg of galacturonic acid obtained in this way is the content of one-tenth of the weight of the washed and dried sample. To calculate the percent galacturonic acid on a moisture- and ash-free basis, multiply the number of mg obtained by 1000/x, in which x is the weight, in mg. of the washed and dried sample.

Note: If the Pectin is known to be of the non-amidated type, only V_1 and V_2 need to be determined, and V_3 may be regarded as zero.

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in chemistry or related fields are intended to be within the scope of the following claims.

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CLAIMS

1. A fat replacer comprising a pectin composition, wherein the pectin composition comprises at least a population of pectin which is covalently cross linked.

2. Use of a pectin composition as a fat replacer, wherein the pectin composition comprises at least a population of pectin which is covalently cross linked.

3. Use of a pectin composition as an emulsifier in a foodstuff and to partially or completely replace fat in a foodstuff.

4. An emulsifier comprising a pectin composition wherein the pectin composition comprises a least a population of pectin that is covalently cross linked.

5. Use of a pectin composition as an emulsifier wherein the pectin composition comprises a least a population of pectin that is covalently cross linked.

6. The invention of any one of the preceding claims wherein the pectin composition is obtainable from or is obtained from sugar beet.

7. The invention of any one of the preceding claims wherein the pectin composition is obtained by contacting an oxidase with a pectin material.

8. The invention of claim 7 wherein the oxidase is laccase.

9. The invention of claim 8 wherein the laccase is a laccase as described in WO 96/03440.

10. The invention of claim 7, 8 or 9 wherein the pectin material is obtainable from or is obtained from sugar beet.

11. A foodstuff comprising a fat replacer according to claim 1.

12. A foodstuff comprising an emulsifier according to claim 4.

13. A foodstuff according to claim 11 or 12 wherein the foodstuff is selected from dairy products (including milk products and ice cream), low fat spread, mayonnaise, yoghurt, sauces, meat and meat products, poultry products, fish products, fruit spreads, bakery products including bakery fillings, margarine, reconstituted fruits, jams, fruit preparations, fruit fillings, ripples, fruit sauces, stewed fruit, and fine foods (such as dressings including salad dressings; ketchup, vinaigrette dressings and soups) and beverages, including drinking yoghurt, a fruit juice, beverage concentrate and fruit based beverages.

14. A process for preparing a foodstuff, the process comprising the step of incorporating a pectin composition in the foodstuff, wherein the pectin composition comprises at least a population of pectin which is covalently cross linked, as a fat replacer to partially or completely replace fat.

15. A fat replacer substantially as hereinbefore described with reference to the Examples.

16. An emulsifier substantially as hereinbefore described with reference to the Examples.

17. A foodstuff substantially as hereinbefore described with reference to the Examples.

18. Use of a pectin composition as a fat replacer substantially as hereinbefore described with reference to the Examples.

19. Use of a pectin composition as an emulsifier substantially as hereinbefore described with reference to the Examples.

20. A process substantially as hereinbefore described with reference to the Examples.

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/IB 00/00051

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23L1/0524

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 41547 A (DU PONT) 24 September 1998 (1998-09-24) column 7, line 36 -column 8, line 10 column 9, line 9 - line 38	1-20
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 April 2000	26/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Vuillamy, V

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INTERNATIONAL SEARCH REPORT

Inte. onal Application No
PCT/IB 00/00051

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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X	GB 2 311 024 A (SYSTEMS BIO IND) 17 September 1997 (1997-09-17) page 2, line 19 -page 5, line 10 ------	3

3

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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